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ON THE PINEAL AREA AND ADJACENT STRUCTURES OF THE BRAIN OF THE DIPNOAN FISH, *PROTOPTERUS ANNECTENS* (OWEN)

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INTRODUCTION

The morphology of the pineal area of the dipnoan fish, *Protopterus annectens*, so far has not been described. In connection with an investigation of the central nervous system of *Protopterus annectens*, however, Burckhardt (1890, 1892) gave a brief description of the dorsal region of the brain, indicating the presence of a pineal organ and, interior to that organ, a conspicuous structure which he named coronarium. Because of the taxonomical position of *Protopterus*, and the considerable variation in the morphology of the pineal structures, especially among teleost fishes (Studnička 1905, Tilney and Warren 1919, Friedrich-Freska 1932, Rasquin 1958 and others), it seemed of interest to describe the dorsal differentiations of the brain of this dipnoan fish, with special reference to the pineal area, and to compare them with the corresponding structures of teleosts.

Material and methods. Four specimens of *Protopterus annectens* fixed in Bouin's solution, and one specimen in formol-alcohol, were investigated. Two heads were sectioned, one transversely and one sagittally, at 50 microns, using celloidin technique. In the others, the brain was dissected out and cut in paraffin at 7 microns. The celloidin sections were stained in Mallory's

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phosphotungstic acid haematoxylin (Romeis 1948, p. 163), and the paraffin sections were stained in Gomori's aldehyde fuchsin (Gabe 1953) and in Mallory's triple stain.

Terminology. Studnička (1905, p. 4) defined the pineal area as the dorsal parts of the diencephalon from the "commissura posterior to the paraphysis." This definition has been adopted in this study. Structures situated anterior of the velum transversum are generally called paraphysis. The latter belongs morphologically to the telencephalon (Kappers 1957, p. 52). In his description of the central nervous system of *Protopterus annectens,* Burckhardt (1892) called the conspicuous structure anterior of the velum transversum, the coronarium, probably because of its vascular pattern. However, this structure should be called paraphysis (Studnička 1905, p. 4). The present study has confirmed Studnička's opinion, for the structure satisfies all the criteria of a true paraphysis cerebri, mainly because of its position in front of the saccus dorsalis and the velum transversum but also because of its general structure. The terminology is in agreement with that discussed by Kappers (1956).

DESCRIPTION

No pineal spot is associated with the pineal area as found in certain teleosts, amphibians and lizards. The general morphology of the pineal brain area is shown in Figure 1. It includes a number of well-defined structures, namely: an organon subcommissuralis, underlying a conspicuous commissura posterior, a commissura habenulae, a pineal organ, and a saccus dorsalis.

The organon subcommissuralis (Fig. 5) consists of columnar, ciliated, secretory cells containing granular Gomori-positive material as in teleosts and other vertebrate classes (Wingstrand 1953). The pineal organ, arising from the commissura posterior area, consists distally of an end-vesicle which is attached to the brain by means of a proximally divided stalk. The pineal organ extends forward and overlies both the saccus dorsalis and the paraphysis and is situated close to the skull roof (Figs. 1, 2). The end-vesicle did not penetrate the skull in my material (cf. Wiedersheim 1893).

The histology of the vascularized pineal organ does not differ markedly from that found in teleost fishes, in which mainly two

types of cells have been observed: the sensory cells and the supporting cells. The primary sensory cells (N. Holmgren 1918, 1920, Friedrich-Freksa 1932, Rasquin 1958, and others) in *Protopterus*, as in most teleosts, can be differentiated from the supporting cells ("Stützzellen," Friedrich-Freksa 1932) which show a strong affinity for the acid fuchsin of Mallory's stain especially the hyperchromatic nuclei of the cells. The cytoplasm of the supporting cells is reduced and the cell membranes seem almost to enclose nuclei. However, compared to teleost fishes,

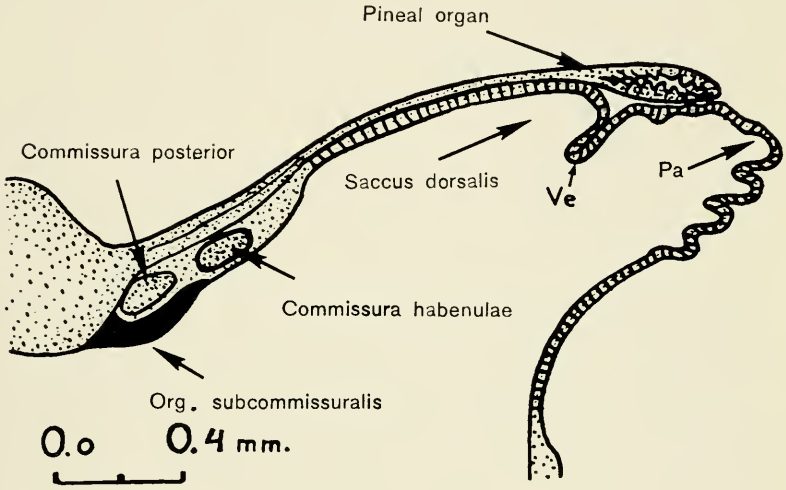

Fig. 1. Schematical picture of the pineal area and adjacent structures of the dipnoan fish, *Protopterus annectens*. The conspicuous paraphysis (*Pa*) is situated anterior to the velum transversum (*Ve*).

there are very few supporting cells present in the pineal organ of *Protopterus*. The primary sensory cell-type has a well defined nucleus with nucleoli, and the cytoplasm had a distal process similar to that of the amphibian sensory cell-type (N. Holmgren 1918, Bargmann 1943). The sensory cells in teleosts have been found to be secretory in nature (N. Holmgren, 1920). Since the pineal sensory cells resemble both those of the teleosts and the amphibians and there is a significant decrease in supporting

glial elements of the pineal organ, one is tempted to regard the pineal of *Protopterus* as a type intermediate between that of teleosts and amphibians.

No parapineal organ or rudiment of it could be found in any of the five specimens. A parapineal rudiment present in the adult has so far not been described in teleost fishes. A parapineal organ is, however, always present in the teleost embryo, but it degenerates in the adult.

Anterior to the pineal organ, the commissura habenulae is present in the dorsal roof of the diencephalon (Fig. 5), marking the limit between the parts of the diencephalon which posteriorly give origin to the pineal- and parapineal complex, and the parts which anteriorly form the saccus dorsalis and velum transversum.

In the commissura habenulae area, it is observed that the right nucleus habenulae is larger than the left one in two specimens, and of about the same size in one specimen. Similar variation has been observed in teleosts. Gierse (1904, p. 618) and Handrick (1901, p. 7) reported asymmetry between the habenular nuclei in certain deep sea teleosts. Such individual variation within one species was frequently found in a number of teleosts (N. Holmgren 1920); this fact already had been pointed out by Gierse (1904), who found that sometimes the nucleus habenulae dexter, sometimes the nucleus habenulae sinister was the larger. The significance of this asymmetry has been extensively discussed in the literature (Gaskell 1890, Friedrich-Freksa 1932, and others). The saccus dorsalis which surrounds the pineal stalk consists of neuroependym, which corresponds to the observations in teleosts (Studnička 1905, Friedrich-Freksa 1932). The anterior limit between the saccus dorsalis and the paraphysis is formed by a not very well defined velum transversum (Fig. 1), which indicates the anterior end of the pineal area.

The paraphysis, shown in Figures 3 and 6, is very much folded and vascularized. Vessels with blood corpuscles are observed between the folds (Fig. 6) of the paraphysis, where also aldehydefuchsin-positive material was observed (S).

Although the specificity of the aldehydefuchsin stain is not well established so far, it may be of interest to note that similar staining reactions were observed in the same slides in the subcommissural organ and also in the nucleus preopticus secretion of the hypothalamus.

The descriptions and comparisons presented in this paper have shown that there is no considerable difference between the pineal morphology of *Protopterus* and that of teleost fishes. The pineal organ of *Protopterus* resembles that of primitive teleosts, especially that of the Clupeidae and Salmonidae. The pineal region of *Protopterus* also resembles the pineal area of other dipnoans such as *Ceratodus* (Huxley 1876, Studnička 1905, and N. Holmgren and v. der Horst 1925). A well defined paraphysis cerebri has previously been observed in another dipnoan, *Ceratodus*, and in the chondrostean *Acipenser* (Studnička 1905). In selachians, on the other hand, the paraphysis, although present during the early ontogeny, is not found in the adult (Kappers 1957). In teleosts the conditions are essentially similar, although the paraphysis is present in a number of species (U. Holmgren 1959).

The resemblance in the histology of the pineal (as described above) between *Protopterus* and the amphibian is not surprising, considering the taxonomic position of the lungfish. The presence of aldehydefuchsin-positive material in the paraphysis has been dealt with by Scharrer (1951), who described the presence of such material in the paraphysis of certain snakes. This secretory material of the snake arose from the nucleus paraventricularis area of the diencephalon. The described aldehydefuchsin-positive granules in *Protopterus* were 2-3 times larger than red blood cells and appeared in close association with blood vessels. Their structure did not resemble that of blood elements. The origin and nature of the aldehydefuchsin-positive material could not be determined on the limited material at hand.

SUMMARY

The pineal area of *Protopterus annectens* was studied in five specimens. Its morphology resembles that of primitive teleost fishes. The histology of the pineal cells, on the other hand, is similar to that of amphibians.

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